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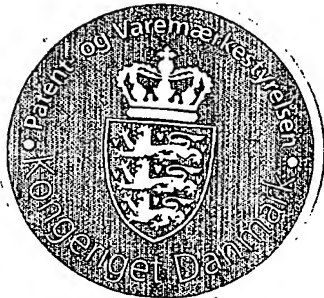
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Title: Stabilization of granules.

IPC: -

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Bo Z. Tiedemann


PATENT- OG VAREMÆRKESTYRELSEN

27 JAN. 2003

PVS

TITLE: STABILIZATION OF GRANULES**FIELD OF THE INVENTION**

The present invention relates to granules comprising active components and acidic buffer components. The acidic buffer components serve the purpose of stabilising the active component containing granules by neutralizing hostile alkaline materials in the environment. The invention further relates to processes of the manufacturer of said granules.

BACKGROUND OF THE INVENTION

It is known to the art to incorporate active components into dry solid particles or granules and thereby protect the active components from inactivation and/or protect the environment from the active component. It is further known to the art to incorporate stabilizers into the active containing particle to protect the active component against inactivation caused by aggressive materials in the environment.

Known prior art related to stabilization of active components in dry granules are:

US 5,858,952 discloses an enzyme-containing granulated product comprising an enzyme and one or more stabilizers selected from the group consisting of reducing agents and antioxidants.

US 5,972,669 discloses methods to improve the processing and storage stability of dry enzyme preparations. To this extend an inorganic salt, e.g. MgSO_4 , is dissolved in an enzyme containing solution which is subsequently dried, using e.g. spray drying.

WO 91/17235 discloses granules containing enzymes and an enzyme protecting agent.

EP 206,417 discloses enzyme granular compositions comprising an enzyme core and alkaline buffer salt coating.

Measures to prevent the reduction of enzyme activity of enzyme-containing granulated products are disclosed by Japanese Patent Application Laid-Open (kokai) No. 62-79298, which describes an enzyme composition in which the core part containing an enzyme is coated with a protective layer containing an alkaline buffering salt of pH 7-11, and by Japanese Patent Application Laid-Open (kokai) No. 3-149298, which describes a bleaching agent containing hydrazine particles, in which the enzyme nuclei are coated with a protective agent such as a water-soluble alkali metal silicate, a transition metal, or a reducing agent.

WO 99/32595 discloses enzyme granules comprising an enzyme core and a hydrated barrier material.

WO 99/32612 discloses granules comprising a protein mixed together with a salt, wherein the granules are prepared by fluid bed.

SUMMARY OF THE INVENTION

Reasons for formulating active components into particles, such as preparing enzyme
5 granules include protection of the active component by separating it from the surrounding potentially hostile environment until the moment when the active component is to be used in an application.

The object of the present invention is to provide stabilized solid formulations of active components, particularly formulations showing improved stability of the active component in hos-
10 tile alkaline environments.

Some active components are sensitive to alkaline compounds and get unstable in their presence, e.g. some enzymes become unstable when getting into contact with alkaline components.

Hence the object of the invention is to improve solid formulations of active components
15 with respect to stabilisation in alkaline environment.

We have surprisingly found that this stability problem can be solved by means of controlling the pH in the environment surrounding the active component by adding acidic buffer components to the granules.

Hence the present invention provides in a first aspect a granule comprising an active
20 component and at least 10% w/w of acidic buffer component capable of neutralizing hostile alkaline compounds present in the environment, wherein said acidic buffer component has a pH of 1 to below 7 when measured as a 10 % aqueous solution and a pK_a in the range of 4 to 9, and wherein the core comprises an active component.

The invention further provides methods for preparation of the granules and
25 compositions comprising the granules.

BRIEF DESCRIPTION OF DRAWINGS

No figures.

DETAILED DESCRIPTION OF THE INVENTION

Introduction

30 The stability of granules comprising active components is influenced by the surrounding environment upon storage, being chemical or physical factors decreasing the stability. Alkaline components have been found to deteriorate the stability of granules comprising active components, in particular granules comprising proteins such as enzymes.

The alkaline component might work directly as a destabilizer of the active component or indirectly by having a positive effect on unwanted reactions such as oxidation processes.

5 By adding acidic buffer components to the granules comprising the active components we have found that we are able of preventing the active components in getting unstable in alkaline environments. The acidic buffer components work by neutralizing the hostile/unwanted alkaline components.

10 A major part of granules comprising active components comprise a core containing the active component and one or more coatings. In very alkaline hostile environments it can be an advantage both to include acidic buffer components in the core and in the coating. Some active components e.g. enzymes get deactivated in acidic environments it is therefore important to investigate how acidic the environment can be before the active components begin to deactivate and therefore it is important to adjust the pH in the granule accordingly. We have found that a way to solve the above mentioned problem, is by either adding less acidic buffer components to the core comprising the active component and more acidic buffer components to the coating or
15 adding acidic buffer components with a not too low pH in solution to the core comprising the active component while adding acidic buffer components with lower pH in solution to the coating, whereby being able of regulating pH in the granule in a way not stressing the active components. By using an acidic buffer component in the core which is less acidic than the acidic buffer component in the coating the amount of the buffer component in the core may be increased.
20

In a particular embodiment of the present invention more than 20 % w/w of the total amount of acidic buffer component present in the granule is present in the core, in a more particular embodiment of the present invention more than 40 % w/w of the total amount of acidic buffer component present in the granule is present in the core, in a most particular embodiment more than
25 60 % w/w of the total amount of acidic buffer component is present in the core.

In a particular embodiment of the present invention the acidic buffer component in the core has a pH of 4 to below 7 and the acidic buffer component in the coating has a pH of 1 to 5. In a further particular embodiment of the present invention the pH of the acidic buffer component used in the core is 4.5 to below 7, in another particular embodiment of the present invention the pH of the acidic buffer component used in the coating is less than 4.5. An example is to
30 use sodium hexametaphosphate (with a pH in a 10% solution of around 6.5) or disodiumhydrogencitrate (with a pH in a 10% solution of around 4.8) in the core and NaH_2PO_4 or KH_2PO_4 (with a pH in 10% solution of around 4.2) in the coating. The acidic buffer component used in the core may even comprise some buffer capacity towards the acid from the coating.

By adding acidic buffer components both to the core and the coating the buffer capacity of the granule is increased. In a further particular embodiment of the present invention the acidic buffer component in the core and in the coating is the same.

5 In less alkaline hostile environments a possibility is to only add acidic buffer components to the core or only add acidic buffer components to the coating.

We have further found that acidic buffer components work very well as regulating agents for solubility of the granules. An important feature of many granules comprising active components is their solubility and release time of the active components. In many applications it is important that the active components are released at a specific desired point in time. By using acidic
10 buffer components with different solubility properties in the coating and in the core it is possible to regulate and control the release time of the active components to a given environment, e.g. making a delayed release or a slow release or a combination thereof. In a particular embodiment of the present invention the acidic buffer component used in the core is less soluble or having a slower solubility rate in aqueous environment compared to the acidic buffer compo-
15 nent used in the coating, typically giving a slow release of the active component (compared to using the more soluble or faster soluble acidic buffer both in the core and in the coating). An example is to use $\text{Ca}(\text{H}_2\text{PO}_4)_2$ which has a solubility of less than 2% in water at 10°C in the core and NaH_2PO_4 which has a solubility around 40% at 10°C in the coating. In another particular embodiment of the present invention the acidic buffer component used in the core is more
20 soluble or having a faster solubility rate in aqueous environment compared to the acidic buffer component used in the coating, typically giving a delayed release of the active component (compared to using the more soluble or faster soluble acidic buffer both in the core and in the coating). An example is to use $\text{Ca}(\text{H}_2\text{PO}_4)_2$ which has a solubility of less than 2% in water at 10°C in the coating and NaH_2PO_4 which has a solubility around 40% at 10°C in the core. Solu-
25 bility properties can also be changed by using polyacrylic acid copolymers where the solubility and solubility rate can be varied almost arbitrarily by the use of different monomers (e.g. hydrophilic or hydrophobic) and varying the ratio between the used monomers.

Acidic buffer component

In the context of the present invention the term "acidic buffer component" is to be un-
30 derstood as a component which is at least partly on its acid form, which can neutralize alkaline compounds and thus comprise a buffer capacity towards an increase in pH. With alkaline compounds in the present invention is understood components which has a pH higher than 8 in a 10% w/w aqueous solution. The acidic buffer component of the present invention may be any acidic buffer component or mixture of acidic buffer components, accordingly, when reference is
35 made to "acidic buffer component" this will in general be understood to include combinations of one or more acidic buffer components.

The suitable acidic buffer component of the present invention has a pH of less than 7 when measured in a 10% aqueous solution. In a particular embodiment of the present invention the acidic buffer component has a pH of 1 to below 7, in a more particular embodiment the acidic buffer component has a pH of 3 to below 7, in a most particular embodiment the acidic buffer component has a pH of 4 to 5.

Furthermore the acidic buffer component of the present invention has a pK_a of 4 to 9. In a particular embodiment of the present invention the pK_a is 5 to 8, in a more particular embodiment the pK_a of the acidic buffer component is 5 to 7. To utilize most of the potential buffer capacity the pH of an aqueous solution is in general below the pK_a .

pK_a is defined as

$$pK_a = -\log K_a$$

wherein K_a is the acidity constant. See T. W. Graham Solomons, Fundamentals of Organic Chemistry, fourth edition, p. 92 to p. 94, (ISBN 0-471-30562-6).

The pH of the acidic buffer component is measured as 10% w/w aqueous solution of the acidic buffer component.

To obtain the best protection of the active component it is important that the granules comprise enough buffer capacity to withstand the hostile alkaline components in the environment. It is found that at least 10 % w/w of the granule has to be acidic buffer component. In a particular embodiment of the present invention the granule comprises more than 25 % w/w of acidic buffer component, in a more particular embodiment the coating comprises more than 40 % w/w of acidic buffer component, in an even more particular embodiment the coating comprises more than 50 % w/w of acidic buffer component.

Particular suitable acidic buffer components of the present invention are salts of H_3PO_4 e.g. NaH_2PO_4 , KH_2PO_4 , and $Ca(H_2PO_4)_2$, polyphosphates e.g. sodium hexametaphosphate, polyacrylic acid and partly neutralized polyacrylic acid and co-polymers thereof, simple organic acids (less than 10 carbon atoms e.g. 6 or less carbon atoms) such as citric acid and salts thereof such as hydrogen citrate, e.g. disodium hydrogen citrate.

In a particular embodiment of the present invention the acidic buffer components are selected from the group consisting of polyacrylic acid and partly neutralized polyacrylic acid and co-polymers thereof, citric acid and Na_2H -citrate.

In an even more particular embodiment of the present invention the acidic buffer components are selected from the group consisting of phosphates such as NaH_2PO_4 , KH_2PO_4 , $Ca(H_2PO_4)_2$ and sodium hexametaphosphate or mixtures thereof.

The acidic buffer components can be present both in the core and in the coating. In a particular embodiment of the present invention there is only acidic buffer component in the core, in a more particular embodiment of the present invention the acidic buffer component is only present in the coating, in a most particular embodiment the acidic buffer component is both present in the core and in the coating. In a further embodiment of the present invention the acidic buffer component in the core and in the coating are different.

In a particular embodiment of the present invention the granule of the present invention comprises a core and a protective coating, wherein said core comprises an active component and said protective coating comprises an effective amount of acidic buffer component having a pH of from about 1 to below 7; said protective coating surrounding said core and said acidic buffer component providing improved stability in the presence of hostile alkaline compounds.

In another particular embodiment of the present invention the granule of the present invention comprises a core and a coating wherein the core comprises an active component and an acidic buffer component with a pH above 4 when measured as a 10 % w/w aqueous solution and said coating comprises an effective amount of acidic buffer component having a pH of 1 to below 7 when measured as a 10 % w/w aqueous solution; said protective coating surrounding said core and said acidic buffer component providing improved stability in the presence of hostile alkaline compounds.

In a more particular embodiment of the present invention the granule comprises a core and a coating wherein the core comprises an active component and an acidic buffer component with a pH above 4 when measured as a 10 % w/w aqueous solution and said coating comprises an effective amount of acidic buffer component having a pH of 3 to below 7 when measured as a 10 % w/w aqueous solution; said coating surrounding said core and said acidic buffer component provides improved stability in the presence of hostile alkaline compounds.

The core

The core unit comprises an active component and possibly acidic buffer component. In a particular embodiment of the present invention the core comprises acidic buffer component. In a particular embodiment of the present invention the core comprises at least 25 % w/w of acidic buffer component, in a more particular embodiment the core comprises at least 40 % w/w of acidic buffer component, in an even more particular embodiment the core comprises at least 60 % w/w of acidic buffer component. In a further particular embodiment of the present invention the core comprises 50 to 95 % w/w of acidic buffer component; in a most particular embodiment of the present invention the core comprises 60 to 90 % w/w of acidic buffer component.

The acidic buffer component in the core has in a particular embodiment of the present invention at least a pH of 2 when measured as a 10 % w/w aqueous solution. In a more particular embodiment of the present invention the acidic buffer component in the core has a pH of at least 4 when measured as a 10 % w/w aqueous solution. In an even more particular embodiment the acidic buffer component has a pH of 3 to below 7. In a most particular embodiment the acidic buffer component has a pH of 4 to 6.

Furthermore the acidic buffer component in the core has a pK_a of 4 to 9. In a particular embodiment of the present invention the pK_a is 5 to 8, in a more particular embodiment the pK_a of the acidic buffer component in the core is 5 to 7. To utilize most of the potential buffer capacity the pH of an aqueous solution is in general below the pK_a .

Besides of the active components and the acidic buffer component, mentioned vide supra, the core may be constructed in any way or of any material which provides the desired functional properties of the core, e.g. the core may consist of materials which allow readily release of the active components upon introduction to an aqueous medium.

Any conventional methods and non-active materials may be used to prepare the core. Examples of known conventional cores and materials are, inter alia, described in, US 4,106,991 (in particular), EP 170360, EP 304332, EP 304331, EP 458849, EP 458845, WO 01/25412, WO 97/39116, WO 92/12645, WO 89/08695, WO 89/08694, WO 87/07292, WO 91/06638, WO 92/13030, WO 93/07260, WO 93/07263, WO 96/38527, WO 96/16151, WO 97/23606, US 5,324,649, US 4,689,297, EP 206417, EP 193829, DE 4344215, DE 4322229 A, DD 263790, JP 61162185 A, JP 58179492, PCT/DK01/00627.

The core may besides the active component and acidic buffer component comprise fillers, binders, fiber materials, liquid agents, active component stabilizing agents or active component protective agents e.g. antioxidants, cross linking agents, plastizisers, inorganics, dispersing agents, viscosity regulating agents and solubilising agents.

Active components

The active component of the present invention may be any active component or mixture of active components, which benefits from being separated from the environment surrounding the particle. The term "active" is meant to encompass all components, which upon release from the particle upon applying the particle of the invention in a process serve a purpose of improving the process. Suitable actives are those, which are either subject of deactivation and/or causing deactivation to other components in the compositions of the invention.

The active component may be inorganic of nature such as bleach components or organic. Particular active components of the present invention are active biological components such as components obtainable from microorganisms which are usually very sensitive to the

surrounding environment. More particular active components are peptides or polypeptides or proteins. In a most particular embodiment of the present invention the proteins are enzymes.

The enzyme in the context of the present invention may be any enzyme or combination of different enzymes. Accordingly, when reference is made to "an enzyme" this will in general be understood to include combinations of one or more enzymes.

It is to be understood that enzyme variants (produced, for example, by recombinant techniques) are included within the meaning of the term "enzyme". Examples of such enzyme variants are disclosed, e.g., in EP 251,446 (Genencor), WO 91/00345 (Novo Nordisk), EP 525,610 (Solvay) and WO 94/02618 (Gist-Brocades NV).

The enzyme classification employed in the present specification with claims is in accordance with *Recommendations (1992) of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology*, Academic Press, Inc., 1992.

Accordingly the types of enzymes which may appropriately be incorporated in granules of the invention include oxidoreductases (EC 1.-.-.-), transferases (EC 2.-.-.-), hydrolases (EC 3.-.-.-), lyases (EC 4.-.-.-), isomerases (EC 5.-.-.-) and ligases (EC 6.-.-.-).

Preferred oxidoreductases in the context of the invention are peroxidases (EC 1.11.1), laccases (EC 1.10.3.2) and glucose oxidases (EC 1.1.3.4). An Example of a commercially available oxidoreductase (EC 1.-.-.-) is Gluzyme™ (enzyme available from Novozymes A/S). Further oxidoreductases are available from other suppliers. Preferred transferases are transferases in any of the following sub-classes:

- a) Transferases transferring one-carbon groups (EC 2.1);
- b) transferases transferring aldehyde or ketone residues (EC 2.2); acyltransferases (EC 2.3);
- c) glycosyltransferases (EC 2.4);
- d) transferases transferring alkyl or aryl groups, other than methyl groups (EC 2.5); and
- e) transferases transferring nitrogenous groups (EC 2.6).

A most preferred type of transferase in the context of the invention is a transglutaminase (protein-glutamine γ -glutamyltransferase; EC 2.3.2.13).

Further examples of suitable transglutaminases are described in WO 96/06931 (Novo Nordisk A/S).

Preferred hydrolases in the context of the invention are: Carboxylic ester hydrolases (EC 3.1.1.-) such as lipases (EC 3.1.1.3); phytases (EC 3.1.3.-), e.g. 3-phytases (EC 3.1.3.8) and 6-phytases (EC 3.1.3.26); glycosidases (EC 3.2, which fall within a group denoted herein as "carbohydrases"), such as α -amylases (EC 3.2.1.1); peptidases (EC 3.4, also known as proteases); and other carbonyl hydrolases].

In the present context, the term "carbohydrase" is used to denote not only enzymes capable of breaking down carbohydrate chains (e.g. starches or cellulose) of especially five- and six-membered ring structures (i.e. glycosidases, EC 3.2), but also enzymes capable of isomerizing carbohydrates, e.g. six-membered ring structures such as D-glucose to five-
 5 membered ring structures such as D-fructose.

Carbohydrases of relevance include the following (EC numbers in parentheses):

α -amylases (EC 3.2.1.1), β -amylases (EC 3.2.1.2), glucan 1,4- α -glucosidases (EC 3.2.1.3), endo-1,4-beta-glucanase (cellulases, EC 3.2.1.4), endo-1,3(4)- β -glucanases (EC 3.2.1.6), endo-1,4- β -xylanases (EC 3.2.1.8), dextranases (EC 3.2.1.11), chitinases (EC 3.2.1.14), poly-
 10 galacturonases (EC 3.2.1.15), lysozymes (EC 3.2.1.17), β -glucosidases (EC 3.2.1.21), α -galactosidases (EC 3.2.1.22), β -galactosidases (EC 3.2.1.23), amylo-1,6-glucosidases (EC 3.2.1.33), xylan 1,4- β -xylosidases (EC 3.2.1.37), glucan endo-1,3- β -D-glucosidases (EC 3.2.1.39), α -dextrin endo-1,6- α -glucosidases (EC 3.2.1.41), sucrose α -glucosidases (EC 3.2.1.48), glucan endo-1,3- α -glucosidases (EC 3.2.1.59), glucan 1,4- β -glucosidases (EC
 15 3.2.1.74), glucan endo-1,6- β -glucosidases (EC 3.2.1.75), arabinan endo-1,5- α -L-arabinosidases (EC 3.2.1.99), lactases (EC 3.2.1.108), chitosanases (EC 3.2.1.132) and xylose isomerases (EC 5.3.1.5).

Examples of commercially available proteases (peptidases) include Kannase™, Everlase™, Esperase™, Alcalase™, Neutrase™, Durazym™, Savinase™, Pyrase™, Pancreatic
 20 Trypsin NOVO (PTN), Bio-Feed™ Pro and Clear-Lens™ Pro (all available from Novozymes A/S, Bagsvaerd, Denmark).

Other commercially available proteases include Maxatase™, Maxacal™, Maxapem™, Opticlean™ and Purafect™ (available from Genencor International Inc. or Gist-Brocades).

Examples of commercially available lipases include Lipoprime™ Lipolase™, Lipolase™
 25 Ultra, Lipozyme™, Palatase™, Novozym™ 435 and Lecitase™ (all available from Novozymes A/S).

Other commercially available lipases include Lumafast™ (*Pseudomonas mendocina* lipase from Genencor International Inc.); Lipomax™ (*Ps. pseudoalcaligenes* lipase from Gist-Brocades/Genencor Int. Inc.; and *Bacillus* sp. lipase from Solvay enzymes). Further lipases are
 30 available from other suppliers.

Examples of commercially available carbohydrases include Alpha-Gal™, Bio-Feed™ Alpha, Bio-Feed™ Beta, Bio-Feed™ Plus, Bio-Feed™ Plus, Novozyme™ 188, Celluclast™, Cellusoft™, Ceremyl™, Citrozym™, Denimax™, Dezyme™, Dextrozyme™, Finizym™,

Fungamyl™, Gamanase™, Glucanex™, Lactozym™, Maltogenase™, Pentopan™, Pectinex™, Promozyme™, Pulpzyme™, Novamyl™, Termamyl™, AMG™ (Amyloglucosidase Novo), Maltogenase™, Sweetzyme™ and Aquazym™ (all available from Novozymes A/S). Further carbohydrases are available from other suppliers.

5 Fillers

Suitable fillers are water soluble and/or insoluble inorganic salts such as finely ground alkali sulphate, alkali carbonate and/or alkali chloride), clays such as kaolin (e.g. Speswhite™, English China Clay), bentonites, talcs, zeolites, chalk, calcium carbonate and/or silicates.

10 Binders

Suitable binders are binders with a high melting point or no melting point at all and of a non waxy nature e.g. polyvinyl pyrrolidon, dextrans, polyvinylalcohol, cellulose derivatives, for example hydroxypropyl cellulose, methyl cellulose or CMC. A suitable binder is a carbohydrate binder such as Glucidex 21D available from Roquette Freres, France.

15 Fiber materials

Pure or impure cellulose in fibrous form such as sawdust, pure fibrous cellulose, cotton, or other forms of pure or impure fibrous cellulose. Also, filter aids based on fibrous cellulose can be used. Several brands of cellulose in fibrous form are on the market, e.g. CEPO and ARBOCELL. In a publication from Svenska Trämjolsfabrikerna AB, "Cepo Cellulose Powder" it is stated that for
 20 Cepo S/20 cellulose the approximate maximum fiber length is 500 µm, the approximate average fibre length is 160 µm, the approximate maximum fibre width is 50 µm and the approximate average fibre width is 30 µm. Also, it is stated that CEPO SS/200 cellulose has an approximate maximum fibre length of 150 µm, an approximate average fibre length of 50 µm, an approximate maximum fiber width of 45 µm and an approximate average fiber width of 25 µm. Cellulose fibers
 25 with these dimensions are very well suited for the purpose of the invention. The words "Cepo" and "Arbocel" are Trade marks. A preferred fibrous cellulose is Arbocel BFC200. Also synthetic fibres may be used as described in EP 304331 B1 and typical fibres may be made of polyethylene, polypropylene, polyester, especially nylon, polyvinylformate, poly(meth)acrylic compounds.

Liquid agents

30 A liquid agent is used in conventional mixer granulation processes for enabling the build up or agglomeration of the conventional granulating component particles into granules. The liquid agent is water and/or a waxy substance. The liquid agent is always used in a liquid phase in the granulation process but may later on solidify, the waxy substance if present, therefore, is either

dissolved or dispersed in the water or melted. By the term "waxy substance" as used herein is meant a substance which possesses all of the following characteristics 1) the melting point is between 30 and 100°C, preferably between 40 and 60°C, 2) the substance is of a tough and not brittle nature, and 3) the substance possesses a certain plasticity at room temperature. Both water
 5 and waxy substance are liquid agents, i.e. they are both active during the formation of the granules; the waxy substance stays as a constituent in the finished granules, whereas the majority of the water is removed during a drying step.

Examples of waxy substances are polyglycols, fatty alcohols, ethoxylated fatty alcohols, mono-, di- and triglycerolesters of higher fatty, acids, e.g. glycerol monostearate, alkylarylethoxy-
 10 lates, and coconut monoethanolamide.

Active component-stabilizing or active component-protective agents

Active component-stabilizing or -protective agents may fall into several categories: alkaline or neutral materials, reducing agents, antioxidants and/or salts of first transition series metal ions. Each of these may be used in conjunction with other protective agents of the same or different
 15 categories. Examples of alkaline protective agents are alkali metal silicates, -carbonates or bicarbonates which provide a chemical scavenging effect by actively neutralizing e.g. oxidants. Examples of reducing protective agents are salts of sulfite, thiosulfite or thiosulfate, while examples of antioxidants are methionine, butylated hydroxytoluene (BHT) or butylated hydroxyanisol (BHA). Most preferred agents are salts of thiosulfates, e.g. sodium thiosulfate. Also enzyme stabilizers
 20 may be borates, borax, formates, di- and tricarboxylic acids and so called reversible enzyme inhibitors such as organic compounds with sulfhydryl groups or alkylated or arylated boric acids.

Cross linking agents

Cross linking agents may be active component compatible surfactants e.g. ethoxylated alcohols, especially ones with 10 to 80 ethoxy groups.

25 Further suspension agents, mediators (for boosting bleach action upon dissolution of the granule in e.g. a washing application) and/or solvents may be incorporated in the granule core.

In a particular embodiment of the present invention the core is the so called T-granulate wherein active components and granulation material are mixed to form granules incorporating the active components distributed throughout the core such as described in US 4,106,991 e.g.
 30 example 1.

In one particular embodiment the core unit is constructed of a particulate carrier (I) with the active component absorbed, and/or an active component containing layer (II) applied, on the carrier surface, optionally comprising a protecting reducing agent. There may even be additional

coating within the core unit material providing desired functional properties of the core unit material.

The core unit may be in any physical state, such as solid, liquid or gel. In a particular embodiment the core unit is in a solid state.

- 5 As a particular embodiment of the invention the core unit may be prepared by applying an active component containing layer onto a "placebo" carrier (active component free carrier) coated with a layer containing the active component according to the methodology described in e.g. WO 97/39116 or EP 0 193 829. Optionally additional active components may be absorbed into the surface of the carrier.
- 10 In one embodiment of the invention the core unit may be as the core unit described in WO 01/25412. Such core unit may, in terms of its relative mass, comprise up to about 30% w/w, such as up to about 20% w/w, in particular up to about 15% w/w, more particularly up to about 10% w/w, such as up to about 5% w/w of the overall mass of the finished granule.

The coating

- 15 The granule may further be coated with one or more coating layers to provide further improved properties of the granule.

The coating of the present invention may comprise acidic buffer component such as mentioned *vide supra*. In a particular embodiment of the present invention the coating comprises at least 10 % w/w of acidic buffer component, in a more particular embodiment the coating comprises at least 30 % w/w of acidic buffer component, in an even more particular embodiment the coating comprises at least 50 % w/w of acidic buffer component. In a most particular embodiment the coating comprises at least 70 % w/w of acidic buffer component, in a further particular embodiment of the present invention the coating comprises 10 to 100 % w/w of acidic buffer component; in a more particular embodiment of the present invention the coating comprises 30 to 95% w/w of acidic buffer component, in a most particular embodiment of the present invention the coating comprises 60 to 90 % w/w of acidic buffer component.

The acidic buffer component in the coating has in a particular embodiment of the present invention a pH below 5 when measured as a 10 % aqueous solution when measured as a 10 % aqueous solution. In an even more particular embodiment the acidic buffer component in the coating has a pH of 1 to below 7. In a most particular embodiment the acidic buffer component in the coating has a pH of 4 to 5.

Furthermore the acidic buffer component in the coating has a pK_a of 4 to 9. In a particular embodiment of the present invention the pK_a is 5 to 8, in a more particular embodiment the pK_a of

the acidic buffer component in the coating is 5 to 7. To utilize most of the potential buffer capacity the pH of an aqueous solution is in general below the pK_a .

For lowering formation of dust, the coating is preferably substantially free of active components.

- 5 The coating may perform any of a number of functions in the granule, depending on the intended use of the granule. Thus, for example, an additional coating may achieve one or more of the following effects:

(i) further reduction of the dust-formation tendency of a granule without the additional coating according to the invention;

- 10 (ii) further protection of the active compound in the granule against hostile compounds e.g. oxidation by bleaching substances/systems (e.g. perborates, percarbonates, organic peracids and the like);

(iii) dissolution at a desired rate upon introduction of the granule into a liquid medium (such as an aqueous medium);

- 15 (iv) provide a better physical strength of the granule.

Conventional coatings and methods as known to the art may suitably be used, such as the coatings described in US 4,106,991, EP 170360, EP 304332, EP 304331, EP 458849, EP 458845, WO 97/39116, WO 92/12645, WO 89/08695, WO 89/08694, WO 87/07292, WO 91/06638, WO 92/13030, WO 93/07260, WO 93/07263, WO 96/38527, WO 96/16151, WO 20 97/23606, US 5,324,649, US 4,689,297, EP 206417, EP 193829, DE 4344215, DE 4322229 A, DD 263790, JP 61162185 A, JP 58179492.

Besides acidic buffer components the coating may comprise materials selected from binders, fibers, salts, water insoluble minerals, pigments, dyes, enzyme stabilizers or combinations thereof and any granulation materials mentioned vide supra in the section "The core".

25

The coating may further comprise one or more of the following: anti-oxidants, reducing agents, chlorine scavengers, plasticizers, pigments, lubricants (such as surfactants or antistatic agents) and fragrances.

Examples of suitable antioxidants and reducing agents of the present invention are salts of alkali metals and earth alkali metals, salts of sulfite, thiosulfite, thiosulfate, erythorbate, citrate, isopropyl citrate and ascorbate or corresponding acids, silicates, carbonates or bicarbonates, phosphates and nitrit, other suitable materials are methionine, glycine, propyl gallate, tert-butyl hydroquinone, tocopherols, thiodipropionic acid, butylated hydroxytoluene (BHT), butylated hydroxyanisol (BHA) or tannic acid. In particular salts of thiosulfates, e.g. sodium thiosulfate, are suitable.

30

Plasticizers useful in the coating in the context of the present invention include, for example: polyols such as sugars, sugar alcohols, or polyethylene glycols (PEGs) having a molecular weight less than 1000; urea, phthalate esters such as dibutyl or dimethyl phthalate; and water.

5 Suitable pigments include, but are not limited to, finely divided whiteners, such as titanium dioxide or kaolin, coloured pigments, water soluble colorants, as well as combinations of one or more pigments and water soluble colorants.

 As an additional coating layer a lubricant may be applied to the granule. In a particular embodiment the granule comprise an enzyme containing core, a coating and an additional lubricant coating.

 As used in the present context, the term "lubricant" refers to any agent which reduces surface friction, lubricates the surface of the granule, decreases tendency to build-up of static electricity, and/or reduces friability of the granules. Lubricants can also play a related role in improving the coating process, by reducing the tackiness of binders in the coating. Thus, lubricants can serve as anti-agglomeration agents and wetting agents.

 Examples of suitable lubricants are polyethylene glycols (PEGs) and ethoxylated fatty alcohols.

 In appropriate embodiments of granules according to the present invention, the coating layer may be composed as described in US 4,106,991, e.g. with a waxy material such as polyethylene glycol (PEG), optionally followed by powdering with a whitener such as titanium dioxide.

Preparation of the granules

 The invention also relates to a method for the preparation of the active component containing granule described herein.

 In a particular embodiment of the present invention the invention includes a process for preparing granules comprising a core and a coating and at least 10% w/w of acidic buffer component capable of neutralizing hostile alkaline compounds present in the environment, wherein said acidic buffer component has a pH of 1 to below 7 when measured as a 10 % aqueous solution and a pK_a in the range of 4 to 9, and wherein the core comprises an active component.

Preparation of the core

 Methods for preparing the core particles include known enzyme granule formulation technologies e.g. spray drying, fluid bed, fluidized spray drying, spray fluidizing, mixer granulation and

extrusion. Other relevant core particles are layered products, absorbed products, pelletized products, prilled products. The cores may optionally be dried after granulation.

5 Spray dried products, wherein a liquid solution comprising active components e.g. enzymes is atomised in a spray drying tower to form small droplets which during their way down the drying tower dry to form an active component-containing particulate material. Very small particles can be produced this way (Michael S. Showell (editor); Powdered detergents; Surfactant Science Series; 1998; vol. 71; page 140-142; Marcel Dekker).

10 Layered products, wherein the active component e.g. enzyme is coated as a layer around a preformed inert core particle, wherein an active component-containing solution is atomised, typically in a fluid bed apparatus wherein the pre-formed core particles are fluidised, and the active component-containing solution adheres to the core particles and dries up to leave a layer of dry active component on the surface of the core particle. Particles of a desired size can be obtained this way if a useful core particle of the desired size can be found. This type of product is described in e.g. WO 97/23606.

15 In a particular embodiment of the present invention the synthetic polymer and/or the polysaccharide and/or the protein source and/or the antioxidants are mixed together with a liquid e.g. enzyme concentrate or fermentation broth and then atomised onto preformed cores in a coating chamber e.g. a fluid bed, a multistage fluid bed, a fluid bed spray drier optionally with recirculation of fine particles.

20 Absorbed core particles, wherein rather than coating the active component e.g. enzyme as a layer around the core, the enzyme is absorbed onto and/or into the surface of the core. Such a process is described in WO 97/39116.

25 Extrusion or pelletized products, wherein an active component-containing paste is pressed to pellets or under pressure is extruded through a small opening and cut into particles which are subsequently dried. Such particles usually have a considerable size because of the material in which the extrusion opening is made (usually a plate with bore holes) sets a limit on the allowable pressure drop over the extrusion opening. Also, very high extrusion pressures when using a small opening increase heat generation in the active component containing paste, which is harmful to the active component e.g. enzyme. (Michael S. Showell (editor); Powdered detergents; Surfactant Science Series; 1998; vol. 71; page 140-142; Marcel Dekker). In a particular embodiment of the present invention the synthetic polymer and/or the polysaccharide and/or the protein source and/or the antioxidants are mixed together with a liquid e.g. enzyme concentrate or fermentation broth and then extruded.

30 Prilled products, wherein an active component powder e.g. enzyme powder is suspended in molten wax and the suspension is sprayed, e.g. through a rotating disk atomiser, into a cooling chamber where the droplets quickly solidify (Michael S. Showell (editor); Powdered detergents; Surfactant Science Series; 1998; vol. 71; page 140-142; Marcel Dekker). The product obtained is one wherein the enzyme is uniformly distributed throughout an inert material instead of being con-

centrated on its surface. Also US 4,016,040 and US 4,713,245 are documents relating to this technique

Mixer granulation products, wherein an active component-containing liquid is added to a dry powder composition of conventional granulating components. The liquid and the powder in a suitable proportion are mixed and as the moisture of the liquid is absorbed in the dry powder, the components of the dry powder will start to adhere and agglomerate and particles will build up, forming granulates comprising the active component. Such a process where the active component is enzyme is described in US 4,106,991 (NOVO NORDISK) and related documents EP 170360 B1 (NOVO NORDISK), EP 304332 B1 (NOVO NORDISK), EP 304331 (NOVO NORDISK), WO 90/09440 (NOVO NORDISK) and WO 90/09428 (NOVO NORDISK). In a particular product of this process wherein various high-shear mixers can be used as granulators, granulates consisting of the enzyme, fillers and binders etc. are mixed with cellulose fibres to reinforce the particles to give the so-called T-granulate. Reinforced particles, being more robust, release less enzymatic dust.

Methods for preparing a visco-elastic liquid core particle include those described in WO 02/28991 and is hereby incorporated by reference.

In a further particular embodiment of the present invention the cores are prepared by a method comprising:

- a) Preparing a particulate carrier material,
- b) introducing a liquid medium comprising an active component by atomization of the liquid medium into the fluid bed, so as to deposit nonvolatile components including the active component of the liquid medium as an active component containing layer on the carrier, and
- c) removing volatile components of the liquid medium from the cores.

The particulate carrier material may in a preferred embodiment comprise a binder (such as Glucidex™ 21D, from Roquette Freres), a fibre material (such as cellulose fibres) and a filler (such as finely ground sodium sulfate and/or kaolin). The particulate carrier may as well be prepared/granulated and dried as described in Example 1 in US 4,106,991. Following granulation the dry particulate carrier may suitably be sieved, and fractionated after size to obtain a uniform carrier size. In a preferred embodiment the carrier material comprise sugar and/or starch cores, nonpareils and inorganic salt particles or crystals e.g. Na₂SO₄ particles. Preferred carrier sizes measured as the diameter of the carrier are between 0.1-2 mm, e.g. 0.3 - 1.0 mm.

As a further particular embodiment additional active component may be absorbed on the particulate carrier prior to applying the active component layer (II), this absorption may be achieved by:

- a) absorbing the component(s) into the surface of the carrier by contacting the particulate carrier with a liquid comprising the active component in a mixer,
- b) mixing the composition by means of mixing blades, and

- c) drying the active component loaded carrier by fluidising it in a fluid bed apparatus.

Conventional mixing equipment can satisfactorily be used to mix the particulate carrier with the active component-containing liquid medium. The mixing equipment can be a batch mixer or a continuous mixer, such as a convective mixer, see, e.g. Harnby et al., *Mixing in the Process Industries*, pp. 39-53 (ISBN 0-408-11574-2). Non-convective mixing equipment, e.g. rotating drum mixers or so-called pan-granulators, may also be employed.

Drying of active component-loaded particulate carrier, application of the active component containing layer (II) the coating (III) and any additional coatings may be performed in any type of fluidising equipment (such as in a fluid-bed apparatus or other form of fluidizing equipment, such as a Hüttlin-type fluidizer). For a description of suitable fluid-bed equipment, see, e.g., Harnby et al., *Mixing in the Process Industries*, pp. 54-77 (ISBN 0-408-11574-2).

15 Preparation of the coating

Conventional coatings and methods as known to the art may suitably be used, such as the coatings described in Danish PA 2002 00885, Danish PA 2002 00473, WO 89/08694, WO 89/08695, 270 608 B1 and/or WO 00/01793. Other examples of conventional coating materials may be found in US 4,106,991, EP 170360, EP 304332, EP 304331, EP 458849, EP 458845, WO 97/39116, WO 92/12645A, WO 89/08695, WO 89/08694, WO 87/07292, WO 91/06638, WO 92/13030, WO 93/07260, WO 93/07263, WO 96/38527, WO 96/16151, WO 97/23606, WO 01/25412, WO 02/20746, WO 02/28369, US 5879920, US 5,324,649, US 4,689,297, US 6,348,442, EP 206417, EP 193829, DE 4344215, DE 4322229 A, DE 263790, JP 61162185 A and/or JP 58179492.

25 The coating may be prepared by the same methods as mentioned vide supra in the section "Preparation of the core".

Methods for production of the active component containing granule of the present invention may comprise the following steps:

- 30 a) mixing active component and acidic buffer component containing cores with a liquid coating medium comprising a water soluble compound and,
- b) removing volatile components of the liquid medium from the mixture, so as to deposit the nonvolatile components of the liquid coating medium as solid coating layer on the cores.
- or
- 35 a) mixing active component containing cores with a liquid coating medium comprising the acidic buffer component and,

- b) removing volatile components of the liquid coating medium from the mixture, so as to deposit the nonvolatile components of the liquid coating medium as solid coating layer on the cores
- or
- 5 a) mixing active component and acidic buffer component containing cores with a liquid coating medium comprising the acidic buffer component and,
 - b) removing volatile components of the liquid medium from the mixture, so as to deposit the nonvolatile components of the liquid coating medium as solid coating layer on the cores.
- Or
- 10 a) mixing active component and acidic buffer component containing cores with a melted coating medium and,
 - b) cooling to obtain a solid coating layer on the cores.
- or
- a) mixing active component containing cores with a melted coating medium comprising the acidic buffer component and,
- 15 b) cooling to obtain a solid coating layer on the cores
- or
- a) mixing active component and acidic buffer component containing cores with a melted coating medium comprising the acidic buffer component and,
- 20 b) cooling to obtain a solid coating layer on the cores.

In a particular embodiment of the present invention the active component containing granule is produced by a fluid bed process comprising:

- a) fluidising an active component containing core material in a fluid bed apparatus,
- 25 b) introducing a liquid medium comprising a water soluble compound by atomization of the liquid coating medium into the fluid bed, so as to deposit non-volatile components of the liquid medium as a solid coating layer on the core material and,
- c) removing volatile components of the liquid medium from the coated core material.

30 **Compositions comprising the coated particle and their application**

The invention also relates to compositions comprising the granules of the invention. The composition may be any composition, but particularly the compositions are well suited for use in the detergent industry, in the textile industry, in the feed industry and in the baking industry. Accordingly the compositions may be detergent compositions, a feed composition, a baking composition or an additive to be incorporated in such compositions.

Detergents

The coated particles of the invention may be added to and thus become a component of a detergent composition comprising alkaline components.

5 The detergent composition of the invention may for example be formulated as laundry detergent composition for hand or machine washings including a cleaning additive composition suitable for pre-treatment of stained fabrics or a fabric softener composition, or a detergent composition for use in general household hard surface cleaning operations, or a composition for hand or machine dishwashing operations.

10 In a specific aspect, the invention provides a detergent additive comprising the coated particles of the invention. The detergent additive as well as the detergent composition may comprise one or more other enzymes such as a protease, a lipase, a cutinase, an amylase, a carbohydrase, a cellulase, a pectinase, a mannanase, an arabinase, a galactanase, a xylanase, an oxidase, e.g., a laccase, and/or a peroxidase.

15 In general the properties of the chosen enzyme(s) should be compatible with the selected detergent, (i.e. pH-optimum, compatibility with other enzymatic and non-enzymatic ingredients, etc.), and the enzyme(s) should be present in effective amounts.

20 Proteases: Suitable proteases include those of animal, vegetable or microbial origin. Microbial origin is preferred. Chemically modified or protein engineered mutants are included. The protease may be a serine protease or a metallo protease, preferably an alkaline microbial protease or a trypsin-like protease. Examples of alkaline proteases are subtilisins, especially those derived from *Bacillus*, e.g., subtilisin Novo, subtilisin Carlsberg, subtilisin 309, subtilisin 147 and subtilisin 168 (described in WO 89/06279). Examples of trypsin-like proteases are trypsin (e.g. of porcine or bovine origin) and the *Fusarium* protease described in WO 89/06270 and WO 94/25583.

25 Examples of useful proteases are the variants described in WO 92/19729, WO 98/20115, WO 98/20116, and WO 98/34946, especially the variants with substitutions in one or more of the following positions: 27, 36, 57, 76, 87, 97, 101, 104, 120, 123, 167, 170, 194, 206, 218, 222, 224, 235 and 274.

30 Preferred commercially available protease enzymes include EVERLASE™, OVOZYME™, SAVOZYME™, ALCALASE™, SAVINASE™, PRIMASE™, DURALASE™, ESPERASE™, AND KANNAASE™ (Novozymes A/S), MAXATASE™, MAXACAL™, MAXAPEM™, PROPERASE™, PURAFECT™, PURAFECT OXP™, FN2™, and FN3™, FN4™ (Genencor International Inc.).

Lipases: Suitable lipases include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Examples of useful lipases include lipases from *Humicola* (synonym *Thermomyces*), e.g. from *H. lanuginosa* (*T. lanuginosus*) as described in EP 258

068 and EP 305 216 or from *H. insolens* as described in WO 96/13580, a *Pseudomonas* lipase, e.g. from *P. alcaligenes* or *P. pseudoalcaligenes* (EP 218 272), *P. cepacia* (EP 331 376), *P. stutzeri* (GB 1,372,034), *P. fluorescens*, *Pseudomonas* sp. strain SD 705 (WO 95/06720 and WO 96/27002), *P. wisconsinensis* (WO 96/12012), a *Bacillus* lipase, e.g. from *B. subtilis* (Dartois et al. 5 (1993), *Biochemica et Biophysica Acta*, 1131, 253-360), *B. stearothermophilus* (JP 64/744992) or *B. pumilus* (WO 91/16422).

Other examples are lipase variants such as those described in WO 92/05249, WO 94/01541, EP 407 225, EP 260 105, WO 95/35381, WO 96/00292, WO 95/30744, WO 94/25578, WO 95/14783, WO 95/22615, WO 97/04079 and WO 97/07202.

10 Preferred commercially available lipase enzymes include LIPOLASE™ and LIPOLASE ULTRA™ (Novozymes A/S).

Amylases: Suitable amylases (α and/or β) include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Amylases include, for example, α -amylases obtained from *Bacillus*, e.g. a special strain of *B. licheniformis*, described in more de- 15 tail in GB 1,296,839.

Examples of useful amylases are the variants described in WO 94/02597, WO 94/18314, WO 96/23873, and WO 97/43424, especially the variants with substitutions in one or more of the following positions: 15, 23, 105, 106, 124, 128, 133, 154, 156, 181, 188, 190, 197, 202, 208, 209, 243, 264, 304, 305, 391, 408, and 444.

20 Commercially available amylases are DURAMYL™, TERMAMYL™, FUNGAMYL™ and BAN™ (Novozymes A/S), RAPIDASE™, PURASTAR™ and PURASTAR OXAM™ (from Genencor International Inc.).

Cellulases: Suitable cellulases include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Suitable cellulases include cellulases from the 25 genera *Bacillus*, *Pseudomonas*, *Humicola*, *Fusarium*, *Thielavia*, *Acremonium*, e.g. the fungal cellulases produced from *Humicola insolens*, *Myceliophthora thermophila* and *Fusarium oxysporum* disclosed in US 4,435,307, US 5,648,263, US 5,691,178, US 5,776,757 and WO 89/09259.

Especially suitable cellulases are the alkaline or neutral cellulases having colour care benefits. Examples of such cellulases are cellulases described in EP 0 495 257, EP 0 531 372, 30 WO 96/11262, WO 96/29397, WO 98/08940. Other examples are cellulase variants such as those described in WO 94/07998, EP 0 531 315, US 5,457,046, US 5,686,593, US 5,763,254, WO 95/24471, WO 98/12307 and PCT/DK98/00299.

Commercially available cellulases include CELLUZYME™, and CAREZYME™ (Novozymes A/S), CLAZINASE™, and PURADAX HA™ (Genencor International Inc.), and KAC-500(B)™ (Kao Corporation).

5 Peroxidases/Oxidases: Suitable peroxidases/oxidases include those of plant, bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Examples of useful peroxidases include peroxidases from Coprinus, e.g. from *C. cinereus*, and variants thereof as those described in WO 93/24618, WO 95/10602, and WO 98/15257.

Commercially available peroxidases include GUARDZYME™ (Novozymes A/S).

Mannanase: Suitable mannanases include MANNAWAY™ (Novozymes A/S).

10 The detergent enzyme(s) may be included in a detergent composition by adding separate additives containing one or more enzymes, or by adding a combined additive comprising all of these enzymes. A detergent additive of the invention, i.e. a separate additive or a combined additive, may be formulated so as to contain one or more of the particles of the invention comprising different enzymes.

15 The detergent composition of the invention may be in any convenient dry form, e.g., a bar, a tablet, a powder, a granule or a paste. It may also be a liquid detergent, in particular non-aqueous liquid detergent.

20 The detergent composition comprises one or more surfactants, which may be non-ionic including semi-polar and/or anionic and/or cationic and/or zwitterionic. The surfactants are typically present at a level of from 0.1% to 60% by weight.

When included therein the detergent will usually contain from about 1% to about 40% of an anionic surfactant such as linear alkylbenzenesulfonate, alpha-olefinsulfonate, alkyl sulfate (fatty alcohol sulfate), alcohol ethoxysulfate, secondary alkanesulfonate, alpha-sulfo fatty acid methyl ester, alkyl- or alkenylsuccinic acid or soap.

25 When included therein the detergent will usually contain from about 0.2% to about 40% of a non-ionic surfactant such as alcohol ethoxylate, nonylphenol ethoxylate, alkylpolyglycoside, alkyl dimethylamineoxide, ethoxylated fatty acid monoethanolamide, fatty acid monoethanolamide, polyhydroxy alkyl fatty acid amide, or N-acyl N-alkyl derivatives of glucosamine ("glucamides").

30 The detergent may contain 0-65 % of a detergent builder or complexing agent such as zeolite, diphosphate, triphosphate, phosphonate, carbonate, citrate, nitrilotriacetic acid, ethylenediaminetetraacetic acid, diethylenetriaminepentaacetic acid, alkyl- or alkenylsuccinic acid, soluble silicates or layered silicates (e.g. SKS-6 from Hoechst).

The detergent may comprise one or more polymers. Examples are carboxymethylcellulose, poly(vinylpyrrolidone), poly(ethylene glycol), poly(vinyl alcohol), poly(vinylpyridine-N-oxide), poly(vinylimidazole), polycarboxylates such as polyacrylates, maleic/acrylic acid copolymers and lauryl methacrylate/acrylic acid copolymers.

- 5 The detergent may contain a bleaching system, which may comprise a H₂O₂ source such as perborate or percarbonate, which may be combined with a peracid-forming bleach activator such as tetraacetylenediamine or nonanoyloxybenzenesulfonate. Alternatively, the bleaching system may comprise peroxyacids of e.g. the amide, imide, or sulfone type.

- 10 The enzyme(s) of the detergent composition of the invention may be stabilized using conventional stabilizing agents, e.g., a polyol such as propylene glycol or glycerol, a sugar or sugar alcohol, lactic acid, boric acid, or a boric acid derivative, e.g., an aromatic borate ester, or a phenyl boronic acid derivative such as 4-formylphenyl boronic acid, and the composition may be formulated as described in e.g. WO 92/19709 and WO 92/19708.

- 15 The detergent may also contain other conventional detergent ingredients such as e.g. fabric conditioners including clays, foam boosters, suds suppressors, anti-corrosion agents, soil-suspending agents, anti-soil redeposition agents, dyes, bactericides, optical brighteners, hydrotropes, tarnish inhibitors, or perfumes.

- 20 It is at present contemplated that in the detergent compositions any enzyme, may be added in an amount corresponding to 0.01-100 mg of enzyme protein per litre of wash liquor, preferably 0.05-5 mg of enzyme protein per litre of wash liquor, in particular 0.1-1 mg of enzyme protein per litre of wash liquor.

The enzyme of the invention may additionally be incorporated in the detergent formulations disclosed in WO 97/07202, which is hereby incorporated as reference.

25 EXAMPLES

Example 1

Uncoated protease granulates was produced as described in US 4,106,991 example 1 with the following exceptions:

- 30 • A small scale high shear mixer (Mi-Mi-Pro granulator from Pro-C-epT) was used in stead of a Lödige mixer
- Finely grinded sodium sulfate was used in stead of sodium chloride as filler material
- Finely grinded buffer salt was added according to the table below

- The enzyme concentrate (added as a liquid) contained also a carbohydrate binder (Avedex W80 dextrin)

The storage stability of the produced granulates in an ADW powder A (stored 7 days at 40°C and 60% RH) was tested:

Buffersalt	% residual activity
None (reference)	40
25% NaH ₂ PO ₄	53
60% NaH ₂ PO ₄	78
25% KH ₂ PO ₄	61
60% KH ₂ PO ₄	84
25% Na ₂ H-citrate	61
60% Na ₂ H-citrate	65

5

Addition of buffer salt increases stability. The best results are obtained with a high content of buffer (>25%) and the phosphates are more efficient than citrate.

Example 2

10 Uncoated Savinase granulates were produced as in example 1 with Na₂SO₄ as filler material and using a Lödige mixer. The uncoated granules were coated in a fluid bed (Hüttlin Kugel-Coater) with 2% sodium thiosulfate (applied from a 14% solution in water). The granules were further coated in the fluid bed with 20% salt (see table below), applied from solutions in water (25% salt, 4% titan dioxide, 1% Avedex W80, water ad 100%). Storage stability was tested as in example 1:

Salt	% residual activity
20% Na ₂ SO ₄ (no buffer capacity)	42
20% NaH ₂ PO ₄ (acidic buffer component)	74
20% Na ₂ HPO ₄ (alkaline buffer)	23

15

It is clear from this example that acidic buffer component improve stability and alkaline buffer decrease stability compared to an "inert" (non-buffering) salt.

Percentages of salt are given as % of the weight of uncoated granulate (giving approx. 16% of the final granulate).

5 Example 3

Uncoated amylase was produced as in example 1 with NaH_2PO_4 as acidic buffer component and using a Lödige mixer. Part of these granules were coated with wax in a Lödige mixer as given in US 4,106,991 example 22 using 8% PEG-4000 and 12% of a 50:50 mixture of kaolin and titan dioxide, another part was coated in a fluid bed with 20% NaH_2PO_4 as in example 2 and subsequently coated with wax as the first part using 4% PEG-4000 and 6% titan/kaolin mix.

The storage stability of the produced granulates in an ADW powder B (stored 6 weeks at 35°C and 80% RH) was tested:

Granulate	% NaH_2PO_4 in total	% residual activity
NaH_2PO_4 in core	39	35
NaH_2PO_4 in core and in coat	55 (40% in core and 15% in coat)	50

15 From this example it can be seen that an increase in buffer capacity (increasing the amount of NaH_2PO_4) by using it both in the core and in the coating increases storage stability.

Example 4

Uncoated amylase was produced as in example 1 with Na_2SO_4 as filler and using a Lödige mixer. Part of these granules (reference) were coated with wax in a Lödige mixer as given in US 4,106,991 example 22 using 8% PEG-4000 and 12% of a 50:50 mixture of kaolin and titan dioxide, another part was coated in a UniGlatt fluid bed with 10% polyacrylic acid (applied as a 20% solution of Sokalan PA30 from BASF in adjusted to pH 5.2 with H_2SO_4) and subsequently coated with wax as the first part using 4% PEG-4000 and 6% titan/kaolin mix.

2% of the different granulates was added to ADW powder A and 2.5 grams tablets were produced on a DIAF tablet press. The stability of the tablets (stored 4 weeks at 35°C and 55% RH) was tested:

Granulate	% residual activity
Reference without buffer	57
10% polyacrylic acid in coating	80

The polyacrylic acid buffer coating increase storage stability of the tablets significantly.

CLAIMS

1. A granule comprising a core and a coating and at least 10% w/w of acidic buffer component capable of neutralizing hostile alkaline compounds present in the environment, wherein said acidic buffer component has a pH of 1 to below 7 when measured as a 10 % w/w aqueous solution and a pK_a in the range of 4 to 9, and wherein the core comprises an active component.
2. The granule according to claim 1, wherein the pH of the acidic buffer component is 3 to below 7.
3. The granule according to claim 1, wherein the pK_a of the acidic buffer component is 5 to 7.
4. The granule according to claim 1, wherein the active component is a protein.
5. The granule according to claim 4, wherein the protein is an enzyme.
6. The granule according to any of the claims 1 to 5, wherein said acidic buffer component is present in the coating.
7. The granule according to any of the claims 1 to 5, wherein said acidic buffer component is present in the core.
8. The granule according to any of the claim 1 to 5, wherein said acidic buffer component is both present in the core and in the coating.
9. The granule according to claim 8, wherein the amount of acidic buffer component present in the core is less than 40 % of the total amount of acidic buffer component present in the granule.
10. The granule according to claim 8, wherein the acidic buffer component in the core and in the coating are different.
11. The granule according to claim 8, wherein the acidic buffer component in the core and in the coating is not the same.
12. The granule according to claim 10, wherein the acidic buffer component in the core has a pH of 4 to below 7 and the acidic buffer component in the coating has a pH of 1 to 5.
13. The granule according to claim 1, wherein the amount of acidic buffer component in the granule is at least 25 % w/w.
14. The granule according to claim 1, wherein the amount of acidic buffer component in the granule is at least 40 % w/w.
15. The granule according to any of the claims 1 to 9, wherein the acidic buffer components are selected from the group consisting of NaH_2PO_4 , KH_2PO_4 , $Ca(H_2PO_4)_2$ and sodium hexametaphosphate or mixtures thereof.

16. The granule according to any of the claims 1 to 9, wherein the acidic buffer components are selected from the group consisting of polyacrylic acid and partly neutralized polyacrylic acid and co-polymers thereof, citric acid and $\text{Na}_2\text{H-citrate}$.

5 17. Use of a granule according to any of the claims 1 to 16, in a detergent composition comprising alkaline components.

18. A process for preparing granules according to any of the preceding claims wherein the granule comprises a core and a coating and at least 10% w/w of acidic buffer component capable of neutralizing hostile alkaline compounds present in the environment, wherein said acidic buffer component has a pH of 1 to below 7 when measured as a 10
10 % aqueous solution and a pK_a in the range of 4 to 9, and wherein the core comprises an active component.

19. The process according to claim 18, wherein the granule is prepared in a mixer, a fluid bed, a fluidized spray dryer, a spray fluidizer, a spray dryer or an extruder.

15

ABSTRACT

This invention relates to A granule comprising a core and a coating and at least 10% w/w of acidic buffer component capable of neutralizing hostile alkaline compounds present in the environment, wherein said acidic buffer component has a pH of 1 to below 7 when measured as a 10 % w/w aqueous solution and a pK_a in the range of 4 to 9, and wherein the core comprises an active component.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/DK2004/000050

A. CLASSIFICATION OF SUBJECT MATTER IPC 7 C12N9/98		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC 7 C12N C07K		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used) EPO-Internal, WPI Data, PAJ, FSTA, BIOSIS		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00/63336 A (LEYENDECKER MARY RUTH ; SADLOWSKI EUGENE STEVEN (US); SONG XIAOQING (U) 26 October 2000 (2000-10-26) the whole document page 15, line 17 - page 16, line 28 -----	1-17
X	US 3 630 930 A (DAVIS JERRY EDISON ET AL) 28 December 1971 (1971-12-28) the whole document -----	1-17
A	WO 00/01793 A (NOVONORDISK AS) 13 January 2000 (2000-01-13) cited in the application page 7, paragraph 15 - page 18, paragraph 33 ----- -/--	
<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex.		
* Special categories of cited documents		
A document defining the general state of the art which is not considered to be of particular relevance *E* earlier document but published on or after the international filing date *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) *O* document referring to an oral disclosure, use, exhibition or other means *P* document published prior to the international filing date but later than the priority date claimed *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. *&* document member of the same patent family		
Date of the actual completion of the international search 13 July 2004		Date of mailing of the international search report 26/07/2004
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016		Authorized officer Piret, B

INTERNATIONAL SEARCH REPORT

International Application No
PCT/DK2004/000050

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
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A	GB 1 415 301 A (UNILEVER LTD) 26 November 1975 (1975-11-26) cited in the application page 2, line 3 - line 62 page 4, line 39 - page 5, line 10 -----	
A	GB 1 395 330 A (UNILEVER LTD) 21 May 1975 (1975-05-21) cited in the application page 1, left-hand column, line 42 - right-hand column, line 75 page 2, left-hand column, line 23 - right-hand column, line 120; example 1 -----	

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Information on patent family members

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